THE COLLECTION AND PRESERVATION OF SAMPLES OF SEWAGE FOR ANALYSIS.

STEPHEN DEM. GAGE AND GEORGE O. ADAMS.

IT is well known that the composition of the sewage from any source is not uniform, varying with the time of day, the amount of surface water entering the sewers, and also with the temperature and with the time which elapses between collection and analysis. The variations in the composition due to these different factors have been frequently discussed, and it is unnecessary to enter into their further consideration at this time.

In the design and control of sewage disposal systems it is of the first importance to obtain the true average composition of each day's flow of sewage, by collecting series of samples covering 24 hour periods. The number of such samples which may be collected is usually limited, however, by the capacity of the testing laboratory to make the necessary analytical determinations before decomposition has started, and also unless exceptional facilities for the transportation of samples are provided, the time elapsing between collection and analysis is sufficient in many cases to affect seriously the accuracy of the results. If some simple and effective method of preserving these samples were used, the capacity of the laboratory to handle a series of samples would be greatly increased, as the work could be extended over several days; and there would also be an increase in the value of the analytical results, since the samples when analyzed would be in the same condition as when collected.

In the control of many municipal sewage areas it is customary to send samples to a central laboratory at stated times, and from the results of the analyses of these samples to interpret the purification accomplished by the area. That these samples are not representative is well known, but hitherto attention has been centered on improving the analytical methods, to the neglect of methods of sampling. Monthly or semi-monthly analyses of average samples, made up of small portions collected two or three times daily and preserved in some manner by the filter attendants, would yield more representa-

tive results without imposing any great burden on the filter attendants.

Two methods of preserving samples are open to us: the use of cold and the use of chemicals. The use of cold, while fairly satisfactory for short periods, is limited by the fact that ice is not readily obtainable at sewage disposal works, is difficult to handle, increases transportation charges, and even when obtainable is less convenient than chemical treatment. It is generally understood that the decomposition which takes place in sewage and in the effluents from sewage filters is largely, if not entirely, due to bacteriolytic action. To be thoroughly effective, then, the chemical employed as a preservative should immediately check all bacterial action, should be comparatively cheap and easily obtainable, and, above all, should not interfere with any of the chemical determinations.

The report of the Committee on Standard Methods of Water Analysis,[1] discussing the time which may be allowed to elapse between collection and analysis of samples, states (p. 14) that six hours is the maximum which may be permitted for sewages and the effluents from sewage purification systems. On p. 15, the report says: "If sterilized by the addition of chloroform, formaldehyde, mercuric chloride, or some other disinfectant, samples for chemical and microscopical examination may be allowed to stand for longer periods. . . . ."

Neither formaldehyde or mercuric chloride conforms to the requirements of an ideal sewage preservative, in that both interfere with certain of the chemical determinations, formaldehyde giving a yellow color with Nessler reagent in the free ammonia determinations and reducing the permanganate of potash in the oxygen consumed process, and mercuric chloride interfering with the determinations of both chlorine and solids. For many years, mercuric acetate has been used at the Lawrence Experiment Station for preserving average samples of sewage; but while the disinfecting action of this has been entirely satisfactory, it is not readily obtained, and interferes with the determination of the solids.

As a result of studies made at the Lawrence Experiment Station during the past year, it has been found that chloroform and carbon-bisulphide most nearly fulfil the ideal conditions. Both are readily

[1] *Jour. Infect. Dis.*, 1905, Supplm. No. 1 p. 1.

obtainable, are sparingly soluble in water, and, being heavier than water, do not evaporate rapidly, requiring only a small amount in each sample. Chloroform, however, is the more satisfactory of the two, being more pleasant to use, thoroughly effective, and, so far as

TABLE 1.

THE RESULTS OF CHEMICAL DETERMINATIONS ON DUPLICATE SAMPLES WITH AND WITHOUT THE PRESENCE OF CHLOROFORM.

	FREE AMMONIA		ALBUMINOID AMMONIA	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter.....	0.0434	0.0426	0.0220	0.0220
" " "	0.9750	0.9500	0.1460	0.1340
Effluent trickling filter.....	0.1120	0.1040	0.0960	0.1020
Effluent contact filter.....	1.0000	1.0800	0.1480	0.1360
" " "	0.6600	0.6800	0.1500	0.1260
Effluent septic tank.....	2.2000	2.2000	0.1900	0.2300
Raw sewage.....	5.3000	5.1000	0.4400	0.4300

NITROGEN AS

	NITRATES		NITRITES	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter.....	2.94	2.94	0.0032	0.0032
" " "	2.94	3.11	0.0028	0.0032
Effluent trickling filter.....	1.51	1.60	0.0040	0.0040
" " "	1.43	1.60	0.0020	0.0020
Effluent contact filter.....	0.02	0.02	0.0000	0.0000
" " "	1.03	1.07	.....	.....

	CHLORINE		OXYGEN CONSUMED	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter.....	10.20	10.20	0.37	0.37
Effluent trickling filter.....	8.42	8.42	1.51	1.60
" " "	7.50	7.50	1.58	1.18
Effluent contact filter.....	10.58	10.58	1.20	1.26
" " "	9.60	9.60	0.82	0.74
Effluent septic tank.....	8.80	8.80	3.64	3.60
" " "	9.20	9.20	1.12	1.30
Raw sewage.....	10.10	10.10	5.45	5.70

ORGANIC NITROGEN (KJELDAHL).

	With Chloroform	Without Chloroform
Effluent sand filter.....	0.2698	0.2936
" " "	0.2936	0.2558
Effluent trickling filter.....	0.1788	0.1779
" " "	0.1878	0.1779
Effluent contact filter.....	0.2731	0.2772
" " "	0.2706	0.2501
Effluent septic tank.....	0.2854	0.2837
Raw sewage.....	0.4400	0.4300

we have been able to determine, not interfering with any of the chemical determinations. Carbon-bisulphide interferes with the determination of nitrates by the aluminum method, and is decomposed by some sewages into hydrogen sulphide or free sulphur, which interferes with some of the chemical determinations, and renders it less effective in preventing bacterial decomposition. In order to prove that chloroform had no effect on the results of the various analytical determinations, many determinations were made of samples with and without the addition of that substance. Some of these comparative determinations are shown in Table 1, from which it is seen that, while the results vary slightly among themselves, they are well within the limits of error of sampling and analysis.

Experiments were then made to determine the proportion of chloroform necessary to destroy the bacteria, or at least to prevent them from multiplying. Various amounts of chloroform were added to bottles of sewage from which the suspended matter had been removed by filtering through paper, and the samples were allowed to stand in the laboratory, determinations of the numbers of bacteria being made at frequent intervals. In these experiments it was found that between 5 and 10 c.c. of chloroform in one gallon of sewage would control the bacteria, this amount making practically a saturated solution. In practice based on further experiments, it has been found advisable to add somewhat more chloroform, and to recover the excess when the analyses are complete. The recovery is easily

TABLE 2.

THE EFFECT OF DIFFERENT AMOUNTS OF CHLOROFORM ON THE BACTERIA IN ONE GALLON OF SEWAGE.
(Bacteria per c.c.)

Elapsed Time	No Chloroform	2 c.c. of Chloroform	5 c.c. of Chloroform	10 c.c. of Chloroform
Start	1,270,000	1,300,000	370,000	10,000
1 day	1,650,000	760,000	3,000	300
2 days	2,200,000	1,160,000	6,500	16
3 "	650,000	1,140,000	75	16
4 "	900,000	1,690,000	2,000	90
5 "	730,000	2,000,000	3,900	75
7 "	830,000	3,050,000	1,600	60
9 "	820,000	7,200,000	22,400	180
11 "	480,000	5,250,000	140	30
15 "	190,000	3,120,000	5,800	5
18 "	80,600	6,840,000	6,800	30
21 "	500,000	10,800,000	108,000	90
28 "	280,000	15,120,000	4,600	100
35 "	380,000	21,650,000	90	42
42 "	14,000	6,770,000	28,600	32
49 "	71,400	14,040,000	19,200	75
63 "	24,500	2,250,000	280	60

made by inverting the samples over a funnel in another bottle filled with water, when the chloroform sinks into the lower bottle, and may be distilled when a sufficient amount has accumulated. The effectiveness of different proportions of chloroform in controlling bacterial life is shown in Table 2.

As a method of preservation would, in practical use, be subjected to considerable variation in temperature, experiments were made to determine the effectiveness of chloroform in preserving samples which were allowed to stand at different temperatures. Samples of sewage, from which the suspended matter had been removed by filtering through paper, were treated with chloroform and incubated at 10°, 20°, and 30° C., respectively, analyses being made at frequent intervals. The results of the various determinations fluctuated more or less, but no regular change appears to have taken place in any of the samples, and it is probable that the fluctuations were due to errors in obtaining the proper sample from the bottle and in the chemical methods. The most noticeable change was in the oxygen consumed, and part of this may have been due to oxidation during the numerous shakings incident to removing samples for 13 analyses. The various analytical results in one experiment with sewage treated with chloroform standing at different temperatures are shown in Tables 3, 4, and 5.

In order to test the applicability of the method to sewages which had been more or less completely purified, experiments were made in

TABLE 3.

SEWAGE TREATED WITH CHLOROFORM STANDING AT 10° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start	5.10	0.44	0.82	43.5	5.45	39,000
1 day	5.30	0.43	0.81	43.6	5.70	0
2 days	4.50	0.43	0.78	45.3	4.60	24
3 "	5.60	0.46	0.74	51.1	5.45	16
4 "	5.60	0.45	0.92	40.1	4.60	3
5 "	5.00	0.44	0.82	44.0	5.30	70
7 "	4.90	0.43	0.78	45.3	4.70	22
10 "	5.20	0.39	0.91	35.2	4.85	41
15 "	4.70	0.42	0.74	46.5	4.75	10
17 "	5.20	0.47	0.84	45.8	4.20	14
22 "	4.80	0.38	0.83	39.5	4.65	16
28 "	4.80	0.42	0.83	35.5	4.85	27
36 "	5.00	0.39	0.67	47.8	4.30	25
42 "	5.70	0.41	0.74	45.4	4.25	25

TABLE 4.

SEWAGE TREATED WITH CHLOROFORM STANDING AT 20° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start.....	5.10	0.44	0.82	43.5	5.45	39,000
1 day.....	4.70	0.42	0.81	42.5	5.20	200
2 days.....	4.60	0.41	0.73	46.0	4.45	26
3 "	5.00	0.45	0.74	50.0	5.35	17
4 "	5.30	0.50	0.94	43.6	4.45	11
5 "	5.50	0.43	0.81	43.6	3.55	20
7 "	5.20	0.45	0.81	45.6	4.60	25
10 "	5.20	0.43	....	....	4.50	28
15 "	5.30	0.41	0.81	41.5	4.60	15
17 "	5.20	0.42	0.85	41.0	4.00	6
22 "	4.80	0.40	0.75	45.9	4.30	20
28 "	4.70	0.44	0.78	46.3	4.55	21
36 "	5.30	0.42	0.68	50.6	4.20	55
42 "	5.20	0.41	0.84	40.0	4.40	28

TABLE 5.

SEWAGE TREATED WITH CHLOROFORM STANDING AT 30° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start.....	5.10	0.44	0.82	43.5	5.45	39,000
1 day.....	5.50	0.45	0.82	45.0	4.45	300
2 days.....	5.10	0.39	0.79	40.7	3.95	26
3 "	4.70	0.42	0.72	47.9	4.30	24
4 "	5.40	0.48	0.84	46.9	4.30	23
5 "	6.20	0.43	0.82	43.1	2.50	30
7 "	5.20	0.40	0.75	43.7	4.30	23
10 "	5.20	0.40	0.88	37.3	4.40	40
15 "	4.90	0.41	0.77	43.6	4.35	2
17 "	4.40	0.48	0.83	47.5	3.75	8
22 "	5.30	0.42	0.89	38.7	3.95	16
28 "	5.60	0.41	0.74	45.4	4.30	17
36 "	5.20	0.44	0.84	43.0	4.00	26
42 "	5.20	0.41	0.78	43.1	4.15	31

which effluents from a sand filter, a trickling filter, a contact filter, and septic tank were treated with an excess of chloroform, to determine whether the destruction of the bacteria by the antiseptic would prevent chemical decomposition, as had been noted in the raw sewages. The same fluctuations occurred in these samples as were previously noted with sewage samples. The variations, however, were greater than in the sewages above, because while the former contained little suspended matter, there was a considerable amount in the latter, making it more difficult to obtain a true sample. The results are shown in the following table:

TABLE 6.

TABLE SHOWING PRESERVATIVE ACTION OF CHLOROFORM ON SEPTIC SEWAGE, AND THE EFFLUENTS FROM SAND, CONTACT, AND TRICKLING FILTERS.

ELAPSED TIME	AMMONIA		ORGANIC NITROGEN (KJELDAHL)	RATIO OF ALBUMINOID NITROGEN TO KJELDAHL NITROGEN	OXYGEN CONS.	NITROGEN AS		BACTERIA PER C.C.
	Free	Alb.				Nitrates	Nitrites	
EFFLUENT SAND FILTER.								
Start.....	0.9500	0.1460	0.2936	40.8	0.42	1.48	0.2000	72,000
4 days.....	0.9750	0.1340	0.2558	42.9	0.96	1.81	0.0000	108,000
8 "	0.8750	0.1400	0.2378	48.3	1.01	1.77	0.0000	200
15 "	0.8400	0.1460	0.2608	45.9	1.05	0.91	0.0600	600
22 "	0.7600	0.1740	0.2698	52.3	1.00	1.18	0.0000	98
32 "	.....	.....	.....	....	....	1.68	0.0000	....
EFFLUENT SAND FILTER.								
Start.....	0.0426	0.0220	.....	....	0.37	2.94	0.0032	1,900
3 days.....	.....	.....	.....	....	0.39	3.11	0.0028	14
10 "	.....	.....	.....	....	0.30	2.69	0.0030	....
17 "	0.0378	0.0208	.....	....	0.39	3.11	0.0028	450
EFFLUENT TRICKLING FILTER.								
Start.....	0.1760	0.1880	0.3378	45.6	1.33	0.91	0.0130	80,000
4 days.....	0.0880	0.2040	0.4478	37.4	1.14	1.09	0.0050	76,000
8 "	0.0880	0.2160	0.3649	48.4	1.28	0.92	0.0006	1,000
15 "	0.1120	0.2160	0.4264	41.6	1.61	0.88	0.0050	1,170
22 "	0.1120	0.2200	0.3813	47.3	1.36	1.01	0.0020	720
32 "	.....	.....	.....	....	....	1.09	0.0014	....
EFFLUENT TRICKLING FILTER.								
Start.....	0.1040	0.1020	0.1779	47.0	1.18	1.60	0.0040	17,500
3 days.....	0.1120	0.0960	0.1878	49.8	1.58	1.43	0.0020	30,000
10 "	0.1320	0.1220	0.1911	52.4	1.36	1.55	0.0032	640
17 "	0.1340	0.1320	0.2075	52.1	1.32	1.48	0.0032	800
EFFLUENT CONTACT FILTER.								
Start.....	0.6800	0.1260	0.2501	41.3	0.74	1.07	0.1900	650,000
4 days.....	0.6600	0.1500	0.2706	45.5	0.82	1.03	0.1500	165,000
8 "	0.5400	0.1720	0.2919	48.3	0.85	0.89	0.0400	26
15 "	0.6400	0.1640	0.2583	52.0	0.87	0.72	0.0800	22
22 "	0.6400	0.1720	0.3050	46.2	0.84	0.84	0.0400	14
32 "	.....	.....	.....	....	....	0.95	0.0400	....
EFFLUENT SEPTIC TANK.								
Start.....	2.20	0.23	0.42	45.0	1.30	....	.....	80,000
4 days.....	2.20	0.19	0.34	45.9	1.12	....	.....	120,000
8 "	2.10	0.17	0.31	44.3	1.30	....	.....	85
15 "	1.90	0.20	0.30	55.2	1.44	....	.....	119
22 "	2.00	0.17	0.30	47.0	1.14	....	.....	143

In the foregoing the most marked percentage change noted was in the nitrites. In order to study these nitrite changes, further experiments were made in which mixtures of sewage and water containing

sodium nitrite were treated with chloroform. In these experiments the nitrites in the samples treated with chloroform were unchanged, which would seem to show that the changes occurring in the nitrites in the effluents noted above were due to chemical, rather than bacterial, action, a view recently advanced by Phelps[1] as the result of his studies of the action of contact filters. The results of one of these experiments are shown in the following table:

TABLE 7.

NITRITES.

	CONTROL		CHLOROFORM	
Elapsed Time	Parts per 100,000	Bacteria per c.c.	Parts per 100,000	Bacteria per c.c.
Start.....	0.0320	30,000	0.0320	30,000
2 days.....	0.0320	86,400	0.0320	55
5 "	0.0040	4,430,000	0.0320	18
9 "	0.0000	5,260,000	0.0320	27
12 "	0.0000	2,710,000	0.0320	50
16 "	0.0000	5,250,000	0.0320	19

It has been frequently stated in the preceding that the fluctuations in the analytical results were within the errors of sampling and analysis. In order to ascertain what these errors would be in actual practice, 10 complete analyses were made of a sample of Lawrence sewage, and 10 determinations of nitrates and nitrites were made on the effluent from a trickling filter which contained a considerable amount of suspended matter. In making these analyses the routine procedure was followed, the work being divided between two analysts. The possible errors are from two sources; those due to sampling, i. e., the error in measuring out the volume for each determination and in obtaining an aliquot portion of the suspended matter in the sample, and those existing in the analytical methods. The sampling errors are variable for the different determinations, since it is necessary to take different volumes of the sewage. In the determination of free and unfiltered albuminoid ammonia in this experiment 5 c.c. of the sewage were used, and, owing to the large amount of suspended matter, it was necessary to measure that volume in cylinders, which are less accurate than flasks or pipettes. In determining the filtered

[1] "Contributions from the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology," *Jour. Infect. Dis.* 1905, Supplm. No. 1, p. 61.

albuminoid ammonia 10 c.c. of the sewage were used, the measurements being made also in cylinders.

The volumes of sewage for the determination of organic nitrogen by the Kjeldahl method were measured in flasks, 20 c.c. of the unfiltered and 25 c.c. of the filtered sewage being taken. Samples of the sewage for oxygen consumed were measured in 25 c.c. flasks, and for chlorine in 50 c.c. flasks. In the determination of nitrates 100 c.c. were measured in a flask, boiled down to 25 c.c., made up to 50 c.c. in a Nessler tube, and 2 c.c. of this, measured in a pipette, diluted to 50 c.c. in a Nessler tube for reading. The nitrite determinations were made on 10 c.c., measured in a pipette and diluted to 50 c.c. in a Nessler tube.

The error in the free ammonia as determined by the average deviation from the mean of the 10 determinations was 5 per cent. The mean error in the albuminoid ammonia determinations was 8 per cent and 8.5 per cent, respectively, for unfiltered and filtered samples. For organic nitrogen determined by the Kjeldahl process, the average deviation from the mean was 2.5 per cent for unfiltered samples and 3.3 per cent for filtered samples. The error of the chlorine determinations was practically negligible, while that of the oxygen consumed process was 2.1 per cent. There was no error in the nitrite determinations, all of the 10 results being identical. The

TABLE 8.

THE VARIATION IN RESULTS OF 10 SEPARATE CHEMICAL ANALYSES.

	Free Ammonia	Sewage						Trickling Filter Effluent	
		Albuminoid Ammonia		Organic Nitrogen (Kjeldahl)		Chlorine	Oxygen Consumed	Nitrogen as,	
		Unfiltered	Filtered	Unfiltered	Filtered			Nitrates	Nitrites
1	6.20	0.86	0.46	1.95	0.80	11.80	4.88	1.00	0.0080
2	6.70	0.98	0.53	1.98	0.95	11.90	4.92	1.15	0.0080
3	7.00	0.98	0.43	2.02	0.85	11.89	4.76	1.19	0.0080
4	6.80	1.04	0.43	2.01	0.87	11.91	4.68	1.09	0.0080
5	7.10	1.12	0.39	1.93	0.93	11.80	4.60	1.09	0.0080
6	7.20	1.14	0.41	1.94	0.93	11.85	4.72	1.11	0.0080
7	6.80	1.12	0.51	2.07	0.99	11.80	4.56	1.07	0.0080
8	5.70	1.06	0.53	1.92	0.92	11.80	4.64	1.09	0.0080
9	7.00	0.92	0.47	1.82	0.92	11.80	4.68	1.09	0.0080
10	6.30	0.92	0.50	2.02	0.92	11.80	4.52	1.07	0.0080
Average	6.68	1.01	0.47	1.97	0.92	11.84	4.70	1.10	0.0080
Maximum	7.20	1.14	0.53	2.07	0.99	11.91	4.92	1.19	0.0080
Minimum	5.70	0.86	0.39	1.82	0.85	11.80	4.52	1.00	0.0080

average deviation of the nitrate results from the mean of the 10 readings was 3.6 per cent. The results of the 10 complete analyses of the sewage and 10 determinations for nitrates and nitrites on the trickling filter effluent are shown in Table 8.

CONCLUSIONS.

It is well known that considerable changes may take place in the composition of sewage and the effluents from sewage filters during short periods, and that the elapsing of a few hours between the time of collection and analysis may seriously affect the results of the analyses. Furthermore, samples collected at occasional intervals from sewers or from the effluents of sewage disposal works do not represent the actual average composition of waters from these sources. To be of the greatest value, samples should be collected at frequent intervals, preserved in some manner to prevent chemical changes, and then mixed in aliquot portions to form an average sample for analysis. Experiments at the Lawrence Station have shown that chloroform is a good preservative to use under these circumstances. From 10 to 25 c.c. of chloroform may be added to a gallon bottle, and small samples of from 100 to 200 c.c., collected daily, may be placed in the bottle, which is tightly stoppered; the average sample so obtained may be analyzed after any stated period without fear of material change in the individual samples of which it is composed. The principle errors which may result from such treatment are a small change in the nitrites, and the difficulty arising from the settling out of suspended matter. The nitrite difficulty may be obviated by taking occasional samples and doing nitrite determinations only. Any objection as to precipitation while the average sample is being collected affecting the determination of soluble and suspended organic matter may be overcome by filtering and preserving a separate portion of the daily sample. If such methods be carried out in practice, it is believed that they will result in a more accurate knowledge of the composition of applied sewages and the effluents from sewage disposal works, and will yield more accurate information as to the purification accomplished by the different methods of sewage disposal.